

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:)	Group Art Unit: 1635
BECKER <i>et al.</i>)	Examiner: Shibuya, M.
Serial No. 09/523,237)	Atty. Docket No. GP068-03.CN1
Filed: March 10, 2000)	
For: KITS FOR AMPLIFYING TARGET)	
NUCLEIC ACID SEQUENCES USING)	
MODIFIED OLIGONUCLEOTIDES)	

DECLARATION UNDER 37 C.F.R. § 1.131

Box Non-Fec Amendment
Commissioner for Patents
Washington, D.C. 20231

Sir:

We, Michael M. Becker, Steven T. Brentano and Mehrdad Majlessi, co-inventors of the above-identified patent application, hereby declare as follows:

1. Prior to August 25, 1995, we conceived of and reduced to practice in the United States modified oligonucleotide primers for use in amplifying target nucleic acid sequences, where the modified oligonucleotide primers contained one or more ribonucleotides having a 2'-O-methyl substitution to the ribofuranosyl moiety. Evidence of this prior conception and reduction to practice can be found in attached Exhibit A, which comprises a set of Steven Brentano's laboratory notebook pages setting forth a study which was conducted to test the efficacy of primers containing 2'-O-methyl substitutions in a transcription-mediated amplification procedure. Although the dates on these pages have been redacted, the study set forth therein was completed in the United States prior to August 25, 1995.

2. The amplification study set forth in Exhibit A included primer sets of both T7 and non-T7 oligonucleotide primers. The T7 primers of this study were 50 bases in length,

Considered KAL 04-12-02

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possessed the same base sequence (taking into account DNA/RNA equivalents), and differed in their structures only as follows: (i) the "T7ArpoHIV4195(-)" primers contained only unmodified deoxyribonucleotides; (ii) the "T7ArpoHIV4195(-)m13" primers contained 37 unmodified deoxyribonucleotides and 13 2'-O-methyl modified ribonucleotides positioned at the 3' most end of these primers; (iii) the "T7ArpoHIV4195(-)m18" primers contained 32 unmodified deoxyribonucleotides and 18 2'-O-methyl modified ribonucleotides positioned at the 3' most end of these primers; (iv) the "T7ArpoHIV4195(-)r13" primers contained 37 deoxyribonucleotides and 13 unmodified ribonucleotides positioned at the 3' most end of these primers; and (v) the "T7ArpoHIV4195(-)r18" primer contained 32 unmodified deoxyribonucleotides with the 18 unmodified ribonucleotides positioned at the 3' most end of these primers. A single non-T7 primer was used in this study, which is identified as the "HIV4116" non-T7 primer.

3. The primers of this study were all tested under essentially identical amplification conditions and at concentrations of 8, 15 or 30 pmol of the T7 primer and 30 pmol of the non-T7 primer in the presence of 5×10^5 copies of an HIV target sequence or in the absence of the HIV target sequence. Following the addition of each primer set to an amplification reaction mixture under amplification conditions and for a period of time sufficient to amplify target sequence present in an amplification reaction mixture, a $1 \mu\text{l}$ aliquot of amplification reaction mixture was removed from each $100 \mu\text{l}$ amplification reaction mixture present in each reaction vessel. The $1 \mu\text{l}$ aliquots of amplification reaction mixture were then added to separate vessels, each containing $100 \mu\text{l}$ of deionized water.

4. Amplified target sequence present in the sample of each reaction vessel (either the remaining $99 \mu\text{l}$ of undiluted amplification reaction mixture or the $101 \mu\text{l}$ of diluted amplification reaction mixture) was then determined using a homogenous format described as the Hybridization Protection Assay (HPA) in the instant application, (see specification at page 5, lines 10-18), and acridinium ester (AE)-labeled probes specific for a target sequence present in the

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amplified HIV target sequence. Each sample received 0.1 pmol of AE-labeled probe and 4 pmol of identical cold probe, creating a competition assay as described in the instant application (see specification at page 10, lines 19-24). Signal from each sample was measured in relative light units (RLUs) using a luminometer.

5. The results of this study are recorded on page 67 of Exhibit A and are separated into various groupings based on the concentration and structure of the T7 primer tested. Those groups based on the concentration of target sequence present in the amplification mixture are identified as follows: (i) "-" represents the absence of target sequence in the amplification reaction mixture; (ii) "500 copies full format" represents the presence of 5×10^5 copies of the HIV target sequence in the amplification reaction mixture prior to amplification and without any subsequent dilution; and (iii) "500 copies 1 μ l" represents the presence of 5×10^5 copies of the HIV target sequence in the amplification reaction mixture prior to amplification, with 1 μ l of the amplification reaction mixture being diluted with 100 μ l of deionized water subsequent to amplification and prior to detection. And designations for the T7 primer structures are presented as follows: (i) fully deoxyribonucleotide T7 primers are designated as "N-8", "N-15" and "N-30"; (ii) T7 primers having 13 3' end 2'-O-methyl modified ribonucleotides are designated as "m13-8", "m13-15" and "m13-30"; (iii) T7 primers having 18 3' end 2'-O-methyl modified ribonucleotides are designated as "m18-8", "m18-15" and "m18-30"; (iv) unmodified T7 primers having 13 3' end ribonucleotides, with the remaining bases being deoxyribonucleotides are designated as "r13-8", "r13-15" and "r13-30"; and (v) unmodified T7 primers having 18 3' end ribonucleotides, with the remaining bases being deoxyribonucleotides are designated as "r18-8", "r18-15" and "r18-30". The second number in each case indicates the amount of T7 primer added to the amplification reaction mixture in pmol. All results are presented in terms of (RLUs) detected.

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6. As the results of this study demonstrate, 2'-O-methyl modified primers can be used to successfully amplify target nucleic acid sequences. This is evidenced, for example, by a comparison of results from samples containing no target sequence with samples including either 2'-O-methyl modified primers or unmodified deoxyribonucleotide primers. (It is noted that the excessive RLU value for the "r18-15" sample under the "-" category on page 67 of Exhibit A would suggest that this sample was contaminated with target sequence.) The results for both the 13 and 18 base modified primers in these tests were very similar.

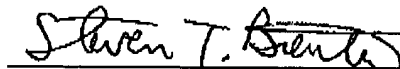
We hereby declare that all statements made herein of our own knowledge are true, and that statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statement may jeopardize the validity of this application and any patent issuing therefrom.

Date: February 4, 2002

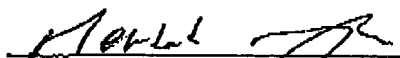
By:


Michael M. Becker, Ph.D.Date: 2-4-02

By:


Steven T. Brentano, Ph.D.Date: 2/4/02

By:


Mehrdad Majlessi

FEB- 6-02 WED 12:17 PM GEN-PROBE

FAX NO. 858 410 8928

P. 14/18

EXHIBIT A

TITLE 2'OME & RNA T7proHIV 4(95E) Primer TestProject No. AMP-T
Book No. 3222

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From Page No. X

Purpose: Test ~~the~~ ^{new} T7proHIV 4(95E) primer w/ either 13 or 18 bases of 2'OME on the 4 or 5' end (see 3222:35283751).
Use lots of target to start. Compare to normal 4(95) primer. Check over all signal & consistency.

Procedure:

- amp w/ all three primers at 8, 15, & 30 μ l
- use HIV 4(16) as non T7 at 30 μ l

	no	oo/ μ l	ng/ μ l	Prod/ μ l
HEB 4(16)	mm 3184-33	17.8 ⁵⁰⁰	0.712 ⁵⁰⁰	43.4 36.9
T7AproHIV 4(16)M13	mm 3189-42	19.8 ⁵⁰⁰	0.712	43.4
T7AproHIV 4(95E)M18	mm 3189-42	19.75	0.712	48.2
T7AproHIV 4(95E)M13	mm 3227-8	6.5	0.26	15.86
T7AproHIV 4(95E)M18	mm 3227-6	9.9	0.396	24.16

all 50 μ l long

- non T7 =

~~HEB 4(16)~~ mm 3097:99 60.1 μ l prod/ μ l (35 μ l HEB aliquot)

- 3 @ & 3 @ amp ea primer, so make mix x 7

- target: use 500 copies ea amp (5 μ l min x 2 amp x 3 μ l = 45 μ l x 60)- for 60 of 50 μ l x 500 copies = 3×10^8 copies in 3 μ l

- target = 2×10^8 copies/ μ l
= 2×10^4 / μ l

→ so use 1.5 μ l + 3 μ l H₂O = 500 copies/50 μ l

no, 2×10^8 / μ l
so used 500,000 copies!

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Witnessed & Understood by me,

MHB

Date

Invented by

Steve Benth

Recorded by

Date

Project No. AMP-TBook No. 3222TITLE cont

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- make premix x165 (10500) at 25/10000

2875 μ l AR (9506061X recomb H₂O)57.4 μ l H₂O (210 μ l for 7000 = 3.49 μ l ea)

- amp mix x7

x7	77 μ l x1	77 μ l x7	77 μ l x7	premix
W-8	8 μ l	56 μ l	1.52 μ l	125 μ l
U-15	15	105	2.75	
U-30	30	210	5.49	
M13-8	8	56	1.29	
M13-15	15	105	2.42	
M13-30	30	210	4.84	
M18-8	8	56	1.16	
M18-15	15	105	2.18	
M18-30	30	210	4.36	
R10-8	8	56	3.53	
R13-15	15	105	6.62	
R13-30	30	210	13.24	
R18-8	8	56	2.32	
R18-15	15	105	4.35	
R18-30	30	210	8.69	

In the
primer pool- Do amp as usual by ^{MTD} days

general
 MTD AR 9506061X
 MTD ER 95030294
 MTD EDB 9505032X

- 25 μ l amp mix/albe (30 30 ea)- 50 μ l target in H₂O

- 60°C 10 min 92°C 5 min

- 42°C Add 25 μ l each rpt (2000u RT, T1)

- 42°C 90 min

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Date

Recorded by

TITLE contProject No. AMP-T
Book No. 3222

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- HPA 1 μ l \pm 9.9 μ l } w/ 4 μ l cold crude
+100 μ l H₂O } 0.1 μ l AB

- probe mix x 160

16 μ l 2x high

16 μ l AB probe = 200 μ l x 80 μ l off μ l

640 μ l cold crude = 1.5 μ l (at 422 μ l/ μ l)

NO! conc of
crude: really
5.45 μ l/ μ l
30 μ l off 15 μ l on

- high 15 min 60°C

- 300 μ l out, DH 10 min 60°C

- H₂O to cool

- read CFTD 2 sec

Results:

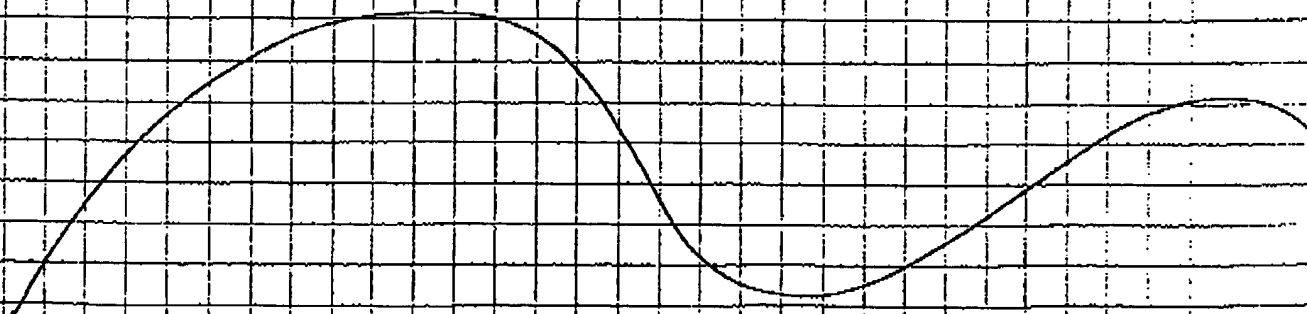
- data next page

- \odot all pretty high, ~10,000 RCU

- no bleach pipets for next time

★ all Normal c RNA c 2' OME primer amp worked
pretty well c had saturated signal even but only

- try again w/ less target

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[Signature]

Date

Invented by

[Signature]

Date

Recorded by

[Signature]

Project No. AMA-TBook No. 3222TITLE Cont

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From Page No. _____

PROTOCOL 18 LISTING

NAME: _____

TYPE: _____

INSTRUMENT: _____

DELAY BETWEEN INSTRUCTIONS: _____

DELAY LAST INSTRUCT TO COUNT: _____

COUNT TIME: _____

BLANK TIME DURATION: _____

NO. OF BLANK TIME REPLICATES: _____

NO. OF SAMPLE REPLICATES: _____

PROTOCOL 18 RAW DATA 2

SOFTWARE REVISION: _____

PROBES SERIAL NUMBER: _____

TYPE: _____

COUNT TIME: _____

NO. OF SAMPLES: _____

OPERATION: _____

SAMPLE 1

1 8548

2 8548

CV= 0.2% AVG 8548

SAMPLE 2

1 18678

2 18678

CV= 0.2% AVG 18678

SAMPLE 3

1 16280

2 16280

CV= 0.2% AVG 16280

SAMPLE 4

1 5620

2 5620

CV= 0.2% AVG 5620

SAMPLE 5

1 6719

2 6719

CV= 0.2% AVG 6719

SAMPLE 6

1 11870

2 11870

CV= 0.2% AVG 11870

SAMPLE 7

1 7216

2 7216

CV= 0.2% AVG 7216

SAMPLE 8

1 8748

2 8748

CV= 0.2% AVG 8748

SAMPLE 9

1 11946

2 11946

CV= 0.2% AVG 11946

SAMPLE 10

1 6887

2 6887

CV= 0.2% AVG 6887

SAMPLE 11

1 9174

2 9174

CV= 0.2% AVG 9174

SAMPLE 12

1 12095

2 12095

CV= 0.2% AVG 12095

SAMPLE 13

1 8489

2 8489

CV= 0.2% AVG 8489

SAMPLE 14

1 271491

2 271491

CV= 0.2% AVG 271491

SAMPLE 15

1 8077

2 8077

CV= 0.2% AVG 8077

SAMPLE 16

1 265897

2 265897

CV= 0.2% AVG 265897

SAMPLE 17

1 264231

2 264231

CV= 0.2% AVG 264231

SAMPLE 18

1 295991

2 295991

CV= 0.2% AVG 295991

SAMPLE 19

1 255740

2 255740

CV= 0.2% AVG 255740

SAMPLE 20

1 264430

2 264430

CV= 0.2% AVG 264430

SAMPLE 21

1 274524

2 274524

CV= 0.2% AVG 274524

SAMPLE 22

1 282820

2 282820

CV= 0.2% AVG 282820

SAMPLE 23

1 274577

2 274577

CV= 0.2% AVG 274577

SAMPLE 24

1 278822

2 278822

CV= 0.2% AVG 278822

SAMPLE 25

1 271703

2 271703

CV= 0.2% AVG 271703

SAMPLE 26

1 281321

2 281321

CV= 0.2% AVG 281321

SAMPLE 27

1 248169

2 248169

CV= 0.2% AVG 248169

SAMPLE 28

1 257414

2 257414

CV= 0.2% AVG 257414

SAMPLE 29

1 252240

2 252240

CV= 0.2% AVG 252240

SAMPLE 30

1 287287

2 287287

CV= 0.2% AVG 287287

SAMPLE 31

1 245471

2 245471

CV= 0.2% AVG 245471

SAMPLE 32

1 226434

2 226434

CV= 0.2% AVG 226434

SAMPLE 33

1 247084

2 247084

CV= 0.2% AVG 247084

SAMPLE 34

1 227048

2 227048

CV= 0.2% AVG 227048

SAMPLE 35

1 246222

2 246222

CV= 0.2% AVG 246222

SAMPLE 36

1 240283

2 240283

CV= 0.2% AVG 240283

SAMPLE 37

1 245814

2 245814

CV= 0.2% AVG 245814

SAMPLE 38

1 245234

2 245234

CV= 0.2% AVG 245234

SAMPLE 39

1 241147

2 241147

CV= 0.2% AVG 241147

SAMPLE 40

1 247088

2 247088

CV= 0.2% AVG 247088

SAMPLE 41

1 245981

2 245981

CV= 0.2% AVG 245981

SAMPLE 42

1 248484

2 248484

CV= 0.2% AVG 248484

PROTOCOL 18 RAW DATA 3

SOFTWARE REVISION: _____

PROBES SERIAL NUMBER: _____

TYPE: _____

COUNT TIME: _____

NO. OF SAMPLES: _____

OPERATION: _____

SAMPLE 1

1 246409

2 246409

CV= 0.2% AVG 246409

SAMPLE 2

1 246409

2 246409

CV= 0.2% AVG 246409

SAMPLE 3

1 246409

2 246409

CV= 0.2% AVG 246409

SAMPLE 4

1 246409

2 246409

CV= 0.2% AVG 246409

SAMPLE 5

1 246409

2 246409

CV= 0.2% AVG 246409

SAMPLE 6

1 246409

2 246409

CV= 0.2% AVG 246409

SAMPLE 7

1 246409

2 246409

CV= 0.2% AVG 246409

SAMPLE 8

1 246409

2 246409

CV= 0.2% AVG 246409

SAMPLE 9

1 246409

2 246409

CV= 0.2% AVG 246409

SAMPLE 10

1 246409

2 246409

CV= 0.2% AVG 246409

SAMPLE 11

1 246409

2 246409

CV= 0.2% AVG 246409

SAMPLE 12

1 246409

2 246409

CV= 0.2% AVG 246409

SAMPLE 13

1 246409

2 246409

CV= 0.2% AVG 246409

SAMPLE 14

1 246409

2 246409

CV= 0.2% AVG 246409

SAMPLE 15

1 246409

2 246409

CV= 0.2% AVG 246409

SAMPLE 16

1 246409

2 246409

CV= 0.2% AVG 246409

SAMPLE 17

1 246409

2 246409

CV= 0.2% AVG 246409

SAMPLE 18

1 246409

2 246409

CV= 0.2% AVG 246409

Witnessed & Understood by me, MAF

Date _____

Invented by _____

Date _____

Recorded by _____

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